

GENITALIC ANALYSIS OF MALES AND FEMALES IN THE
ENCHENOPA BINOTATA (SAY) COMPLEX
(MEMBRACIDAE: HOMOPTERA)

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Abstract.—Male and female genitalia of nine species (8 undescribed, 1 of uncertain identity) of the *Enchenopa binotata* complex were compared to *Campylenchia latipes* and *Enchenopa ignidorsum* to detect diagnostic characters for identification. Discrete character differences in male genitalia of the *E. binotata* complex were noted from *Campylenchia latipes* and *Enchenopa ignidorsum*, but not between members of the complex. These results demonstrate that genitalia are remarkably uniform and provide no practical diagnostic information within the *E. binotata* complex, other than slight size differences apparently related to overall body size. The lack of morphological differentiation of the members of the *E. binotata* complex is discussed in the context of speciation models.

Key Words: Host race, sympatric speciation, *Campylenchia*

The *Enchenopa binotata* (Say) complex of univoltine North American treehoppers, consists of an array of nine biological species (8 undescribed, 1 of uncertain identity) associated with eight genera and nine species of deciduous plants (Wood 1980, Wood in press, Wood and Guttman 1981, 1982, 1983, 1985, Pratt and Wood 1992). These species, in addition to host origin, can be distinguished by differences in allozyme frequencies (Guttman et al. 1981, 1989, Guttman and Weigt 1989, Pratt, Wood, and Datz in prep.), nymphal characters (Pratt and Wood 1992), and adult female pronotal shape and size (Wood and Datz in prep.). Although pronotal shape and size differ among females of the various species, these characters are continuous and not discrete. The apparent lack of external discrete adult characters makes the species difficult to identify and formally describe.

Differences in male genitalia have been successful in providing diagnostic species

characters in the Cicadellidae (Young 1957, Delong and Freytag 1967, Delong 1967) and to some extent in the closely related Membracidae (Broomfield 1976). In general, morphological differences in male genitalia of Membracidae have also been useful in delimiting genera (Caldwell 1949, Kopp and Yonke 1979) or complexes of species (Dietrich and Deitz 1991, McKamey and Deitz 1991). These suggested genitalic characters could be useful to either delimit each presumptive *Enchenopa binotata* species in the complex or subdivide the complex into an array which would facilitate identification and formal description.

METHODS AND MATERIALS

At least six males and five females from each of the following host plants of the *Enchenopa binotata* complex were examined: *Ptelea trifoliata* (Linnaeus), *Juglans nigra* (Linnaeus), *Juglans cinerea* (Linnaeus), *Cercis canadensis* (Linnaeus), *Celastrus*

scandens (Linnaeus), *Liriodendron tulipifera* (Linnaeus), *Robinia pseudoacacia* Linnaeus, *Carya ovalis* (Wang) Sargent, and *Viburnum lentago* Linnaeus. Two individuals of each sex also were examined of *Enchenopa ignidorsum* Walker (Le Selva, Costa Rica) and *Campylenchia latipes* (Say) (Wilmington, Clinton Co., Ohio and Little Orleans, Maryland). [*Campylenchia latipes* was compared to the members of the *E. binotata* complex, since it belongs to a closely related genus and was used as an outgroup in two phylogenetic analyses of the complex (Pratt and Wood, 1992).]

The abdomen from each individual was removed and cleared in 10% NaOH overnight at room temperature. The genitalia were removed and washed in 70% ethanol, and stored in glycerin. One of each species was dissected with sharp tweezers by pulling the styles and aedeagi away from the membranous connective tissue. The male genitalia and dissected parts were affixed with petroleum jelly within glycerin to the bottom of a petri dish and drawn to scale using a camera lucida mounted on a Leica Wild® M10 Stereoscopic microscope (Figs. 1–5).

Genitalia drawn intact were maintained as such for future reference. Their localities by host (Fig. 1) are as follows: A) Lycombine Co., Pennsylvania (code MBL-9) (*Robinia*); B) Greyson, Kentucky (*Cercis*); C) Wooster, Ohio (*Juglans nigra*); D) Wilmington, Clinton Co., Ohio (*Ptelea*); E) Winchester, Virginia (*Viburnum*); F) 3 miles south of Kennet Square, Pennsylvania (*Celastrus*); G) Ithaca, Oswego Co., New York (*Juglans cinerea*); H) Newark, Newcastle Co., Delaware (*Liriodendron*); and I) Wilmington, Clinton Co., Ohio (*Carya*). The localities of dissected specimens (Figs. 2, 3) are the same as above except: A) Harrisburg, Pennsylvania (*Robinia*); B) Wilmington, Clinton Co., Ohio (2-MC-35) (*Cercis*); C) Newark, Newcastle Co., Delaware (*Juglans nigra*); and F) Harrisburg, Pennsylvania (*Celastrus*). Additional specimens were examined from the above localities and from Little

Orleans, Maryland (*Celastrus*) and Nichols Arboretum, Ann Arbor, Michigan (*Ptelea*).

To examine intraspecific variation, aedeagi were dissected as above from four additional specimens of a host associated species (host *Cercis*) (Fig. 5): A) Wilmington, Clinton Co., Ohio (DRD 34); B) Junction of routes 46 and 59, Indiana (MRD 10); C) Saint Louis, Missouri; and D) Ellettsville, Indiana (DRD 37). The specimen from 3B was redrawn as Fig. 5E. Voucher specimens from this study will be deposited in the Entomology Museum at the Smithsonian Institution.

RESULTS

Subtle differences in the shape of the male genitalic structures among the species of the *E. binotata* complex (Figs. 1–3) were due to individual variation and not species differences. Other than slight differences in size of genitalic structures, we were unable to detect any discrete morphological differences in either sex among any of the nine biological species of the *E. binotata* complex. Although the subgenital plate of the species with host *Cercis* (Fig. 1B) is slightly narrower than the others, this character is not discrete, since it will not distinguish it from the species with hosts *Viburnum* (Fig. 1E) and *Celastrus* (Fig. 1F). The genitalia are remarkable in their uniformity.

Size differences in the subgenital plates of the male are probably related to overall body size. The *E. binotata* that use the hosts *Liriodendron* and *Carya*, and *Campylenchia latipes* are generally the largest, whereas the *E. binotata* that use the hosts *Viburnum* and *Ptelea*, and *Enchenopa ignidorsum* are the smallest of the species compared (Figs. 1, 4).

Although the styles of the *E. binotata* complex do not seem to differ from one another (Fig. 2), the anterior arms of the aedeagi do appear to differ (Fig. 3A–I). The member of the *E. binotata* complex which appears to differ most in shape of the aedeagus, is the species which uses *Cercis* as

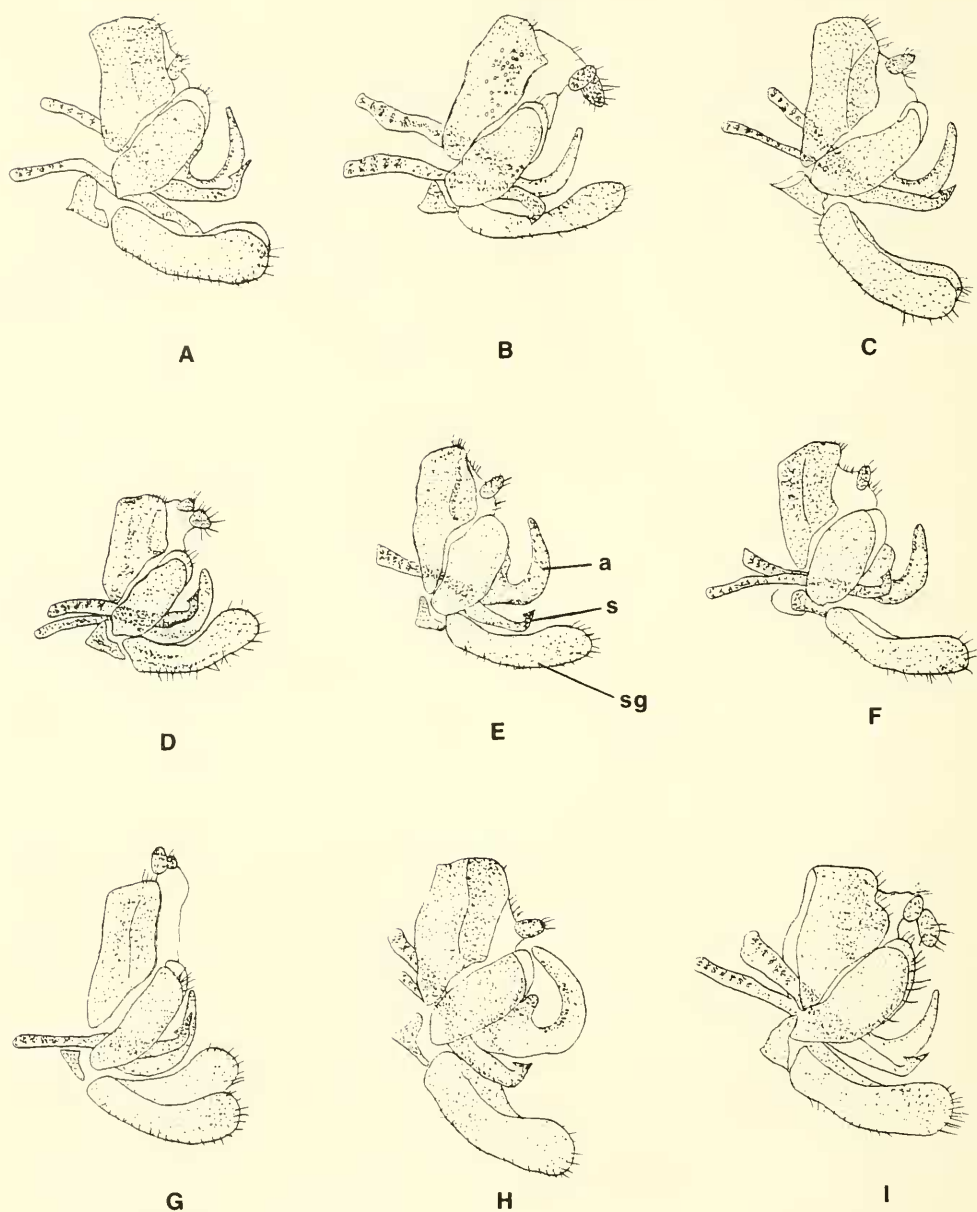


Fig. 1. Male genitalia of species of the *Enchenopa binotata* complex, which are designated by their host A) *Robinia*, B) *Cercis*, C) *Juglans nigra*, D) *Ptelea*, E) *Viburnum*, F) *Celastrus*, G) *Juglans cinerea*, H) *Liriodendron*, and I) *Carya* (all to same scale; the length of the bar is 1 mm). The structures are as follows: sg = subgenital plate, a = aedeagus, s = style.

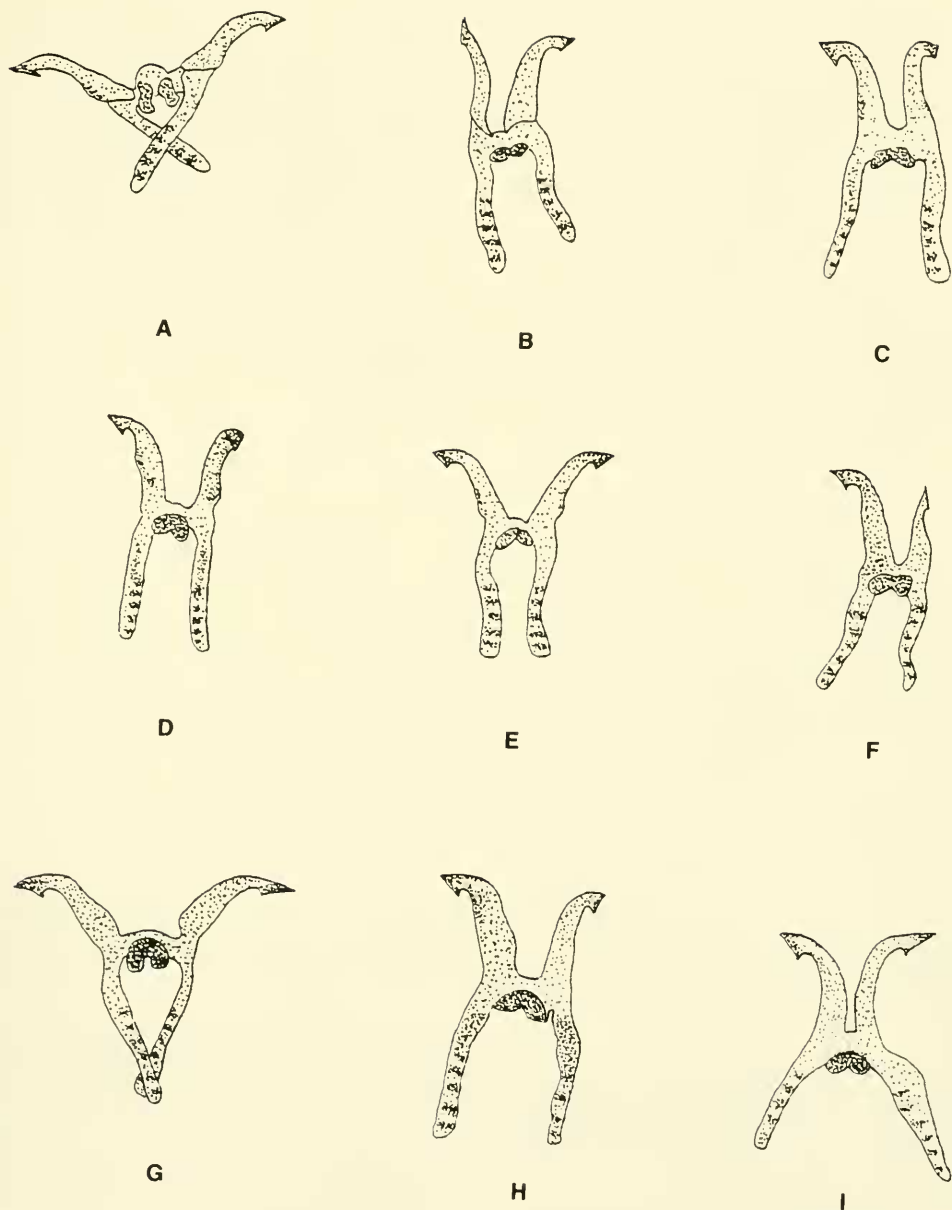


Fig. 2. The dissected styles of male genitalia of species of the *Enchenopa binotata* complex, which are designated by their host A) *Robinia*, B) *Cercis*, C) *Juglans nigra*, D) *Ptelea*, E) *Viburnum*, F) *Celastrus*, G) *Juglans cinerea*, H) *Liriodendron*, and I) *Carya* (all to same scale; the length of the bar is 1 mm).

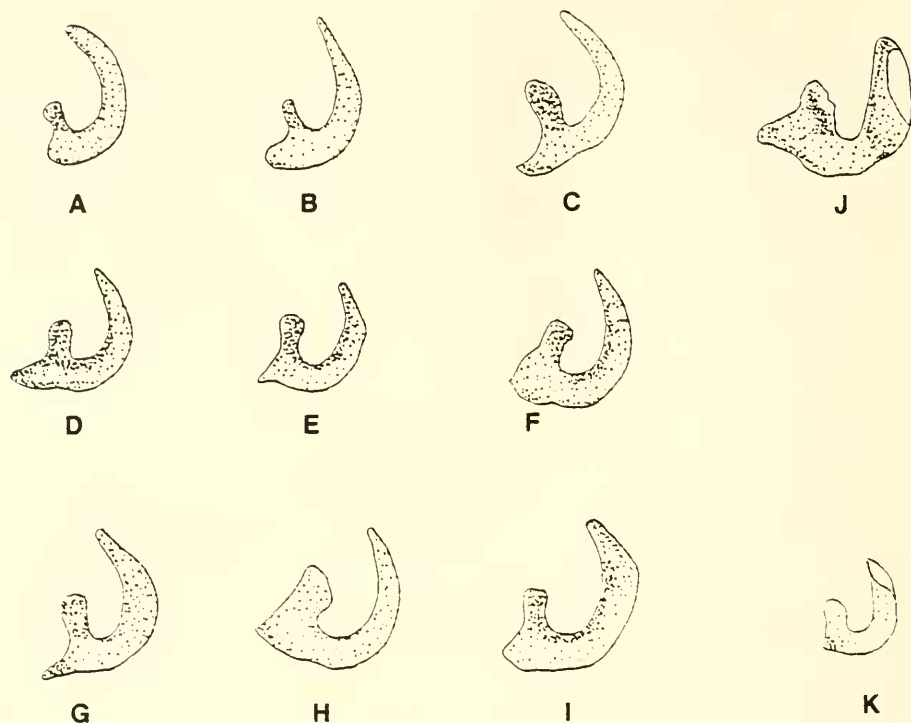


Fig. 3. The dissected aedeagi of male genitalia of species of the *Enchenopa binotata* complex, which are designated by their host A) *Robinia*, B) *Cercis*, C) *Juglans nigra*, D) *Ptelea*, E) *Viburnum*, F) *Celastrus*, G) *Juglans cinerea*, H) *Liriodendron*, and I) *Carya* and J) *Campylenchia latipes*, and K) *Enchenopa ignidorsum* (all to same scale; the length of the bar is 1 mm).

a host; so five individuals of this species were examined to identify intraspecific variation (Fig. 5). The anterior arms of this species seem to vary as greatly as do the species of the *E. binotata* complex.

The male genitalia of *Campylenchia latipes* and *Enchenopa ignidorsum* differ from the *E. binotata* complex in subtle but discrete characters. These include the shapes of the posterior arm of the aedeagi and of the styles (Figs. 2, 3, 4). In *Campylenchia latipes* the posterior arm of the style was bent, whereas all *Enchenopa* were not. In *Enchenopa ignidorsum* the anterior arms of the styles were reduced compared to the other species. On the other hand the female

genitalia of *Campylenchia latipes* and *Enchenopa* species were not found to differ [see Dennis (1952), Fig. 2 for generalized female genitalia]. These observations between *Campylenchia latipes* and the *Enchenopa binotata* complex have been noted previously (Dennis 1952).

DISCUSSION

The lack of diagnostic genitalic characters provides no basis for identification or subdivision of the species complex. Although the members of the *E. binotata* complex seem to differ in the shape of the anterior arms of the aedeagi, these differences are as great intraspecifically as interspecifically

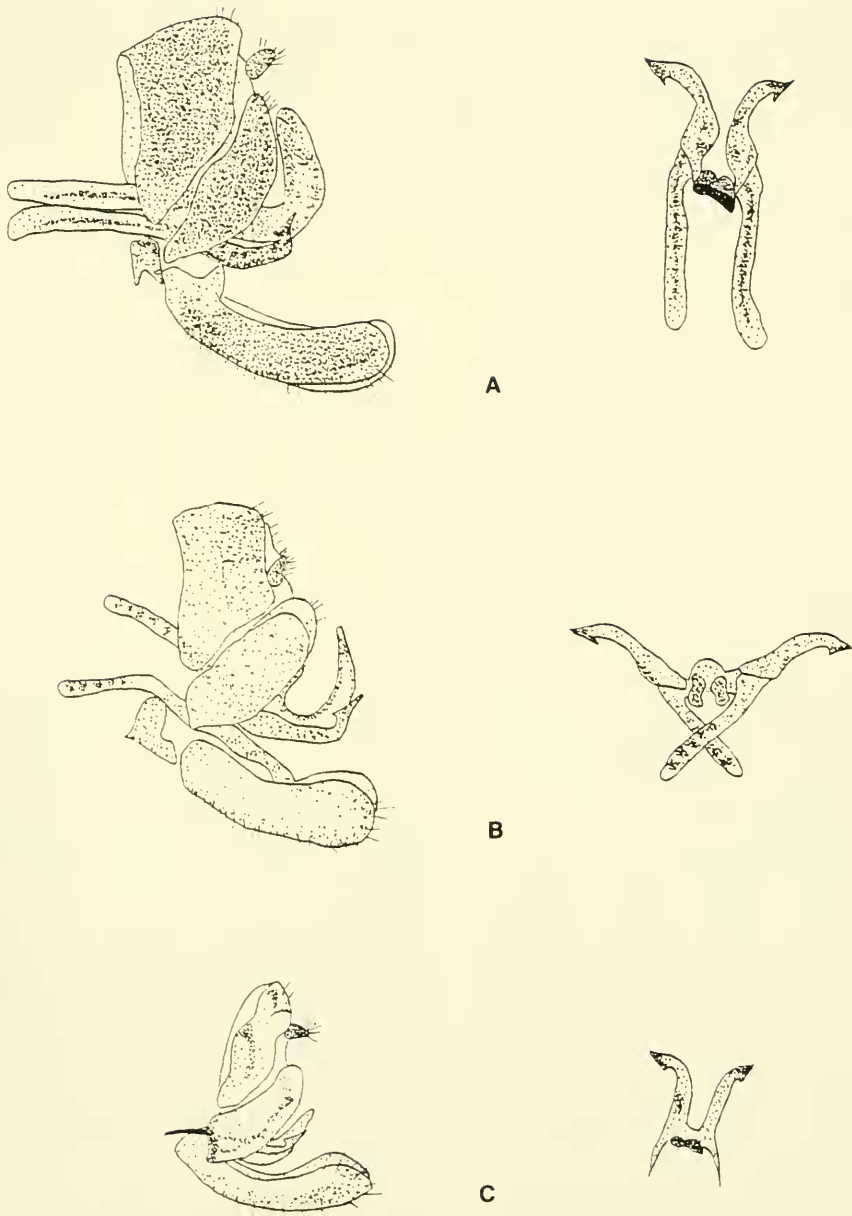


Fig. 4. Male genitalia of A) *Campylenchia latipes*, B) *Enchenopa binotata* host *Robinia* (from Figs. 1A, 2A), and C) *Enchenopa ignidorsum* (all to same scale; the length of the bar is 1 mm).

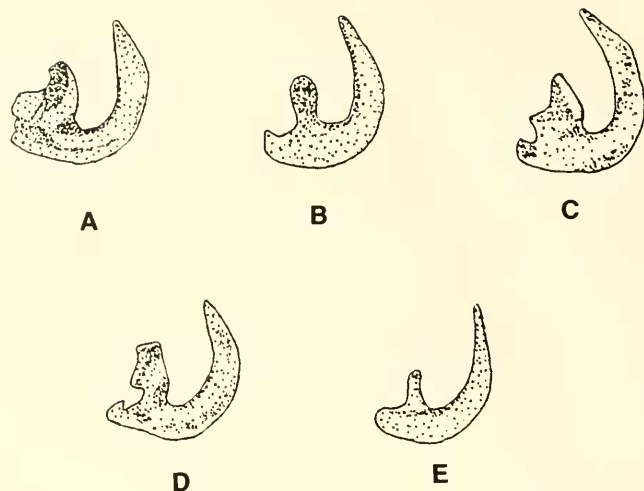


Fig. 5. The dissected aedeagi of 5 *E. binotata* (host *Cercis*), (all to same scale; the length of the bar is 1 mm).

(Figs. 3, 5). Dennis (1960) has also noted similar variation in the anterior arm of another treehopper species. He examined over 1000 male genitalia and found the anterior arm varies greatly, while the posterior arm exhibits little variation.

Even though *Campylenchia latipes* is taxonomically more distant to the *E. binotata* complex than is *Enchenopa ignidorsum*, it seems as similar in male genitalia to the *E. binotata* complex as is *E. ignidorsum*. The only two characters which separate *C. latipes* from the *E. binotata* complex are the bent styles (Fig. 4) and the broad posterior arm of the aedeagus (Fig. 3). *Enchenopa ignidorsum* differs from both *Campylenchia latipes* and the members of the *E. binotata* complex (Fig. 4) by the shortened anterior arms of the styles, the compressed ninth segment, and the long single segmented subgenital plates (the others are composed of two separate segments).

Negative results in general are not reported, but they are of value with the *E. binotata* complex. Clear genitalic differences in this complex could be viewed as

evidence that the members of this complex may have been geographically isolated followed by secondary contact causing selection for genitalic differences as an interspecific reproductive isolating mechanism (Shapiro and Porter 1989). Eberhard (1992) suggests that sexual selection may play an important role in speciation with some insects. The absence of genitalic differentiation within this species complex suggests that selection on these characters has not been intense. If sexual selection has promoted divergence in this complex, it has acted on other structures such as the pronotum.

Because the genitalia of the various members of the *E. binotata* complex differ interspecifically only in size (due to body size), there are probably no mechanical impediments to hybridization among the species associated with the different hosts—except perhaps those due to size. Speciation in these treehoppers has been hypothesized to have occurred through shifts in host plant usage (Wood 1980, Wood and Guttman 1981, 1982, 1983, 1985). In this hypothesis the

primary mode of reproductive isolation in the complex is due to host-induced asynchronous mating or diurnal mating preferences (Wood in press, Wood and Keese 1990, Wood et al. 1990, Wood et al. in prep.). The lack of genitalic differences in the *E. binotata* complex does not support alternative models.

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